Correspondence

Dual capacity of a human olfactory receptor

Marc Spehr[1,4]*, Katlen Schwane[2,4], Stefan Heilmann[3], Günter Gisselmann[2], Thomas Hummel[3] and Hanns Hatt[2]

Certain mammalian olfactory receptor (OR) genes are exclusively or predominantly expressed in spermatozoa [1–2]. One of these receptors, hOR17-4, has been shown to mediate directed chemotactic movement of human sperm *in vitro* [3]. Whether sperm ORs additionally perform their 'conventional' task in olfaction is a longstanding question. Here, we investigate the function of hOR17-4 in human olfaction adopting a molecular, electrophysiological and psychophysical approach. Our results support a model in which the same human OR protein is similarly utilized to fulfil chemosensory functions in such diverse cell types as spermatozoa and olfactory sensory neurons.

Nasal expression of the 'sperm' olfactory receptor hOR17-4 was demonstrated by RT-PCR on human olfactory mucosa cDNA (Figure 1). We identified and mapped distinct exons located upstream of the coding sequence (Fig. 1C) initially postulated by means of bioinformatic analyses. Such upstream exons have been described for a number of human and murine olfactory receptor genes [4]. RT-PCR analysis based on intron-spanning primers proves expression of hOR17-4 in human olfactory epithelium. PCR-products amplified from potential contaminating traces of genomic DNA can be distinguished by size.

In sperm, the most potent hOR17-4 agonist is the odorant bourgeonal, whereas the receptor is antagonized by undecanal [3]. Therefore, we investigated olfactory perception of bourgeonal after brief undecanal exposure. 488 subjects (male and female, 21–37 years of age) were separated into one experimental group, sniffing bourgeonal–undecanal–bourgeonal (BUB) in succession, and various control groups (sniffing either bourgeonal three times (BBB) or a control odorant X–undecanal–odorant X (XUX) in succession). Odorants were presented for five seconds with brief interstimulus intervals (ISI) of 2 s and intensities as well as hedonics were obtained as previously described [5]. Intensity (Figure 1D) and quality (data not shown) ratings after undecanal exposure were then compared to the initial ratings (for the BBB control group responses to the first and the last stimulus were compared). Subjects of the BBB group reported consistent intensities for the three trials (105.1 ± 6.2 %), indicating no sign of adaptation. In contrast, the experimental group (BUB) showed a significant decrease in bourgeonal intensity after brief exposure to undecanal (35.8 ± 2.6%). Such an undecanal dependent effect was not reported by subjects rating the intensity of helional (91.3 ± 9.5%), an odorant structurally related to bourgeonal, geraniol (87.7 ± 7.2%), octanal (104.3 ± 13.9%), isoamyl acetate (99.4 ± 10.1%), vanillin (80.4 ± 24.9%), or eucalyptol (82.2 ± 10.1%). In all groups, no sex-related differences were observed and hedonic ratings changed only slightly after

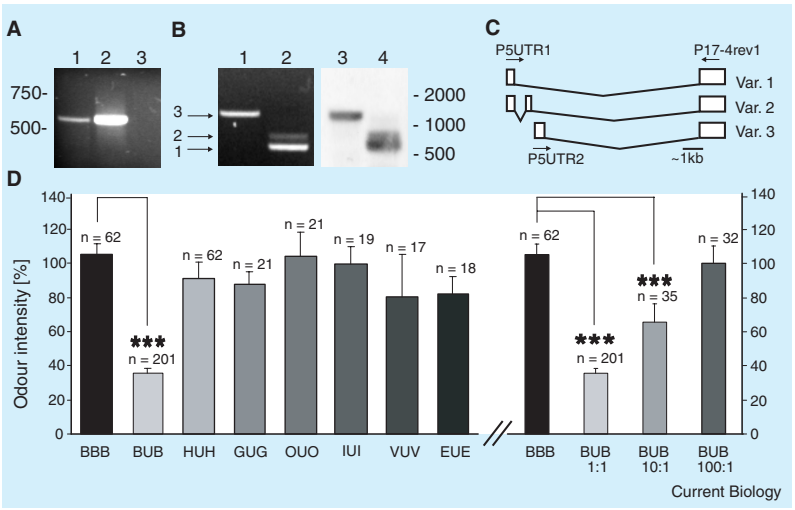

Figure 1. Human sperm odorant receptor hOR17-4 appears to be bi-functional.
(A–C) RT-PCR/Southern blot detection of hOR17-4 expression in human olfactory epithelium. (A) RT-PCR with primers against the hOR17-4 coding region (577 bp); lane 1: cDNA; lane 2: genomic DNA; lane 3: mRNA prior to cDNA synthesis. (B) RT-PCR with intron spanning primers. Candidates for 5'UTR exons were identified using FGENES and FEX software (SoftBerry, Mount Kisco, NY). RT-PCR with primers against two of these predicted exons yielded PCR-products (lane 1: 1120 bp, P17-4rev/P5'UTR2; lane 2: 658 bp and 741 bp, P17-4rev/P5'UTR1) that originated from spliced hOR17-4 mRNA, as shown by Southern hybridization with a hOR17-4 probe (lane 3–4) and by sequencing of the subcloned PCR products. Genomic contamination would yield large fragments > 9 kb that could not be amplified under the given conditions. (C) Exon map of three splice variants of hOR17-4. The upstream exons are located 11207–11062 bp (var.1), 11207–11057 and 9978–9895 bp (var. 2) and 9829–9272 (var. 3) upstream to the hOR17-4 coding exon. (D) The odor intensity of bourgeonal is significantly decreased after brief undecanal pre-exposure (BUB; $p = 4.243 * 10^{-27}$). Perception of the structurally related helional (inactive at hOR17-4 [3]) was unaffected (HUH; $p = 0.063$), just as perception of a broad range of further control odors (geraniol (GUG; $p = 0.061$), octanal (OUO; $p = 0.492$), isoamyl acetate (IUI; $p = 0.299$), vanillin (VUV; $p = 0.086$), eucalyptol (EUE; $p = 0.069$)). Interestingly, undecanal-mediated inhibition of bourgeonal perception depends on the relative concentrations of both odors. While a bourgeonal/undecanal ratio of 10:1 has a weaker, yet still significant, effect on bourgeonal perception (BUB 10:1; $p = 0.0013$), at concentrations 100-times lower than bourgeonal undecanal loses its inhibitory potential (BUB 100:1; $p = 0.677$). Generally, 3 odorants per trial were successively presented in a blinded fashion on paper strips for 5 s each (ISI = 2 s). After a complete trial, subjects were asked to rate odorant intensities using computerized visual analogue scales [5]. Intensities of the third odorant were normalized to the first odorant`s intensity (calculated as 100%; plotted values are means ± SEM).

undecanal exposure, revealing no undecanal related shift in odorant quality among subjects in experimental and control groups (data not shown). Comparable to hOR17-4-mediated behaviors in sperm [3], the effect of undecanal directly depends on the relative concentrations of stimulatory and inhibitory odorants (Figure 1D). Shifting the ratio of bourgeonal and undecanal toward higher agonist concentrations (BUB 10:1, 100:1) gradually diminishes the inhibitory effect of undecanal, potentially indicating a competitive receptor inhibition.

The psychophysical results were confirmed by electro-olfactogram (EOG) recordings [6]. Electrical activity in the olfactory epithelium in response to bourgeonal was dramatically decreased after undecanal exposure (Figure 2). The average bourgeonal-induced field potential was 0.16 ± 0.059 mV under control conditions, whereas three seconds after a short undecanal pulse (1 s) a mean potential of only 0.081 ± 0.041 mV (50.7%; $p = 0.008$) was recorded. In contrast, averaged EOG signals induced by helional (0.139 ± 0.03 mV) proved completely unaffected by undecanal (0.157 ± 0.044 mV) (Figure 2B,C).

In summary, we demonstrate expression of hOR17-4 in the human olfactory epithelium and hypothesize a basically identical receptor operation in both olfactory tissue and spermatozoa. The present study indicates a predominant or even exclusive role of hOR17-4 in olfactory bourgeonal detection or it suggests the inhibition of more bourgeonal-activated human ORs by undecanal. However, undecanal does not affect certain other ORs sensitive to a broad range of odorants (control groups), and a general inhibitory mechanism aside from the receptor level can thus be excluded as well. It seems likely that the antagonistic property of a particular (structurally unrelated) odorant pair represents a common principle of OR activation/inactivation. Thus, design of (odorless) antagonists that specifically target ORs sensitive to unpleasant odors seems feasible in the future.

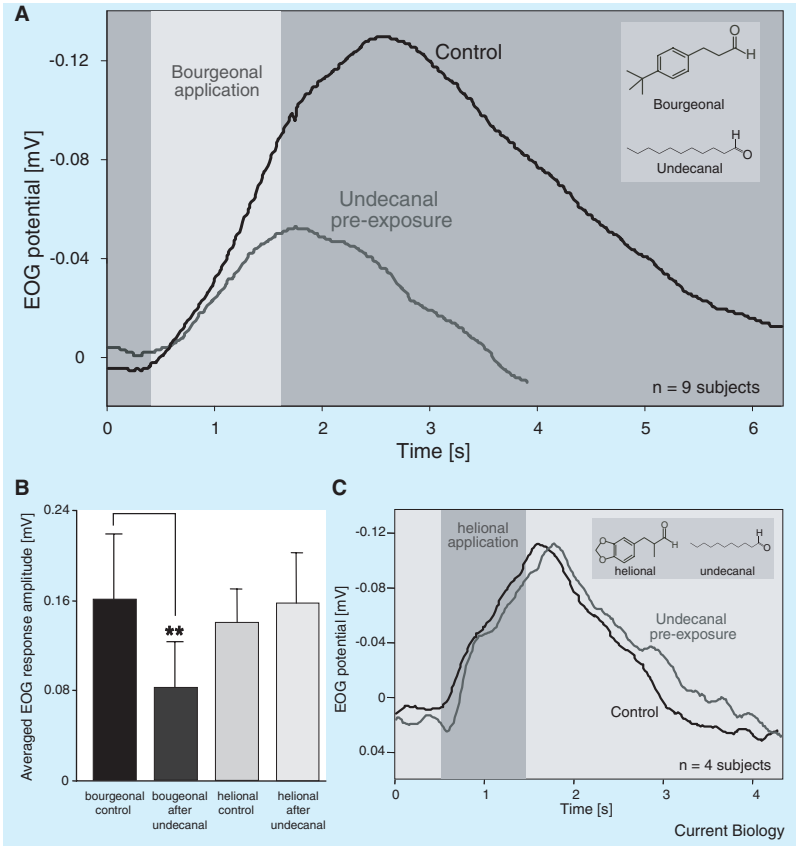

Figure 2. Human electro-olfactogram recordings support the psychometrical findings. Neuronal activity in response to odorant stimulation was recorded extracellularly as described [6]. (A) Averaged responses to bourgeonal (1 s application) of 9 subjects (male and female, 21–29 yrs) are shown before (black) and 3 s after (grey) undecanal exposure (1 s). (B,C) While bourgeonal-dependent electro-olfactogram signals remain strongly inhibited for at least 3 s after a brief application of undecanal, averaged helional-induced field potentials turn out to be unaffected.

Acknowledgments

We thank B.W. Ache, J.A. Riffell, and R.K. Zimmer for comments and discussion. This research was supported by the Heinrich and Alma Vogelsang Stiftung.

References

1. Parmentier, M., Libert, F., Schurmans, S., Schiffmann, S., Lefort, A., Eggerickx, D., Ledent, C., Mollereau, C., Gerard, C., and Perret, J. (1992). Expression of members of the putative olfactory receptor gene family in mammalian germ cells. Nature *355*, 453–455.
2. Vanderhaeghen, P., Schurmans, S., Vassart, G., and Parmentier, M. (1997). Specific repertoire of olfactory receptor genes in the male germ cells of several mammalian species. Genomics *39*, 239–246.
3. Spehr, M., Gisselmann, G., Poplawski, A., Riffell, J.A., Wetzel, C.H., Zimmer, R.K., and Hatt, H. (2003). Identification of a testicular odorant receptor mediating human sperm chemotaxis. Science *299*, 2054–2058.
4. Volz, A., Ehlers, A., Younger, R., Forbes, S., Trowsdale, J., Schnorr, D., Beck, S., and Ziegler, A. (2003). Complex transcription and splicing of odorant receptor genes. J. Biol. Chem. *278*, 19691–19701.
5. Hummel, T., Hummel, C., Pauli, E., and Kobal, G. (1992). Olfactory discrimination of nicotine-enantiomeres by smokers and non-smokers. Chem. Senses *17*, 13–21.
6. Kobal, G., and Hummel, T. (1992). In: The human sense of smell, D.G., Laing, R.L., Doty, and W., Breipohl, eds. (Berlin: Springer press), p. 135.

[1]Department of Anatomy and Neurobiology, University of Maryland, 20 Penn Street, Baltimore MD 21201, USA. [2]Department of Cell Physiology, Ruhr-University Bochum, Universitätsstraße 150, 44780 Bochum, Germany. [3]Smell & Taste Clinic, Department of Otorhinolaryngology, University of Dresden Medical School, Fetscherstraße 74, 01307 Dresden, Germany. [4]Both authors contributed equally to this work
*E-mail: mspeh001@umaryland.edu